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VIRUSES ISOLATED FROM BEAGLE DOGS EXPOSED TO AEROSOLS OF ^{90}Sr and ^{144}Ce

Albuquerque, New Mexico

by

D. L. LUNDGREN, W. E. CLAPPER and A. SANCHEZ

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D. L. Lundgren, W. E. Clapper and A. Sanchez

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ABSTRACT

Many isolations of coxsackie viruses B1, B3 and B5 and echovirus 6, related if not identical to human viruses of the same types were made from nose, throat and rectal swabs of Beagle dogs. Six unidentified enteroviruses were also recovered. Except for the echovirus 6 such isolations have not been previously reported. Three groups of Beagles receiving an aerosol which provided an initial body burden of 35 μCi/kg ^{90}Sr, 90 μCi/kg ^{90}Sr and 140 μCi/kg ^{144}Ce showed no difference in the incidence of coxsackie B viruses from that of controls. However, a significantly greater number of isolations of echovirus 6 and of an unidentified enterovirus were made from controls than from exposed dogs.

Low NT antibody titres against coxsackieviruses B3 and B5 were found in some of the serum from the Beagles that were examined. No titres were observed for coxsackievirus B1, echovirus 6 or one of unidentified enterovirus which were the most frequently isolated. There was no correlation between virus isolation and serum titres.

ACKNOWLEDGMENTS

The authors wish to acknowledge with thanks, the helpful assistance of the Department of Veterinary Medicine in the care and handling of the dogs; the Department of Aerosol Physics for the preparation and administration of the radioactive materials and the Department of Radiobiology for providing the dosimetry. We also wish to express our appreciation to Dr. Roger O. McClellan for helpful suggestions in the preparation of the manuscript.

We are indebted to Dr. Robert M. Chanock, Laboratory of Infectious Diseases, National Institute of Health, Bethesda, Maryland, for the identification of two of these isolates.

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INTRODUCTION

The present study was initiated to determine what viruses occur normally in Beagle dogs and to observe possible changes in their viral flora following inhalation of fission product radionuclides. In previous reports[1, 2] preliminary data on the isolation of coxsackievirus B1 and echovirus 6 from nose, throat and rectal swabs from Beagle dogs before and after exposure to inhalation of radionuclides and from control dogs was presented.

The present report, which includes the preliminary data cited above, consists of the completed studies conducted during a two year period on three groups of dogs exposed to aerosols of ^{90}Sr and ^{144}Ce and control animals. These radionuclides were of interest because they are present in abundant quantities in a nuclear reactor inventory and in the event of a reactor accident could conceivably be released to the local environment.

MATERIAL AND METHODS

Animals

Nine of the Beagle dogs in Group I with the letters FD prefixing their numbers were obtained from a commercial supplier* and 4 were obtained from the School of Veterinary Medicine, University of California, Davis, California. These dogs were received as weaned pups about one year prior to their being used in the present study. All other dogs used were reared in our kennels. During these experiments our

* Mr. Thomas Imlay, Murray, Utah.

colony contained approximately 800 dogs.

Details of the care of these dogs has been published elsewhere[3,4]. In general, compatible pairs of dogs were housed in runs with concrete floors and were separated from other dogs by a cinderblock wall and wire fence. Exceptions to this were that experimental and control dogs were held a month or more in individual metabolism cages after exposure to aerosols of radionuclides. Cages and runs were washed with disinfectant frequently. Access to the kennels was restricted to the caretakers and researchers.

After weaning at 6 weeks of age all dogs were maintained on a diet of 84% dry dog food,* 16% raw meat (USDA)inspected meat; 75% red meat and 25% beef heart and tongue containing not more than 10% fat: This product was fit for human consumption,** and a vitamin-mineral supplement (Vionate).*** Water was available to the dogs at all times.

At weaning time all dogs were injected with anticanine distemper-hepatitis-Leptospira canicola serum of canine origin followed by vaccination and boosters for these agents and for rabies at recommended intervals.

The dogs used were about 13.5 months of age when the studies were initiated. Dogs were selected at random from available litters without regard to sex. The dogs used in the present studies were divided into three groups, each group containing an equal number of controls and dogs exposed to aerosols of radionuclides. The details of inhalation exposure have been published elsewhere.[5]

Group I consisted of 8 controls and 8 experimental dogs which had a mean initial whole body burden of 35 μCi ^{90}Sr/kg with a range of 31 to 46 μCi. Group II was composed of 5 control dogs and 5 dogs which

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* Allied Mills, Chicago, Illinois.

** Karler Packing Company, Albuquerque, New Mexico.

*** E.R. Squibb and Sons, New York, N. Y.

had a mean initial whole body burden of 80 μCi ^{90}Sr/kg with a range of 55 to 127 μCi/kg after exposure. Group III consisted of 6 control dogs and 6 dogs that had a mean initital whole body burden of 140 μCi of ^{144}Ce/kg with a range of 125 to 150 μCi/kg after inhalation exposure.

Specimen collection and preparation

Plastic tubes containing swabs* designed to prevent contamination from the outer area of the anus were used to collect the rectal swabs. Throat and nasal swabs were collected with standard cotton swabs. Immediately after collection swabs were placed in individual tubes containing 2 ml of Hank's balanced salt solution (BSS) with 50 times the concentration of antibiotics used in the tissue culture media. Rectal swabs were collected and stored in stoppered tubes at -70°C until cell cultures were available for inoculation.

Tissue culture systems

The tissue culture systems used for this study were primary rhesus monkey (MK) cells**, Hela cells,*** primary dog kidney (DK) and primary dog lung (DL). The Hela cell cultures were maintained in this laboratory. The DL and DK cell cultures were prepared according to the procedure of Younger[6], with minor modifications, from healthy Beagle pups less than one month of age. The outgrowth media for the Hela, DK and DLcell cultures consisted of 10% calf serum in medium 199 with antibiotics (500 units of penicillin G sodium, 100 ug of streptomycin sulfate, 100 ug of neomycin sulfate and 5 ug Fungizone per ml of media). Confluent monolayers of Hela, DK and DL cell cultures were maintained on a medium consisting of 1% calf serum in Eagle's minimal essential medium (MEM) in Earl's BSS with the same concentration of antibiotics as used in the outgrowth medium. The MK cell cultures were maintained on Eagle's MEM without serum.

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* Falcon Plastics, Los Angeles, California

** Obtained from Shamrock Farms, Inc. Middletown, New York.

*** Original cell lines obtained from Microbiological Associates, Bethesda, Maryland and Department of Microbiology, University of New Mexico School of Medicine.

Virus isolation and identification

Approximately 0.1 ml of the fluid from each swab was inoculated into each of 2 MK, Hela and DK or DL cell culture tubes. For economy of tubes during some of the observations of the Group I dogs, nasal and throat swabs were pooled for inoculation into the MK cell culture tubes. Inoculated tubes were examined for cytopathic effect (CPE) every other day for 7 to 10 days. Negative inoculated cell cultures were frozen at -70°C, thawed and inoculated into fresh cell culture tubes. Three such passages were completed before a sample was discarded as CPE negative.

Positive CPE isolates were passed 2 to 3 times then identified by neutralization (NT) tests with known antisera* in the appropriate cell culture systems. All of the CPE negative 3rd passage MK cell cultures and some of the 3rd passage DK and DL cell cultures were also tested for hemadsorption with guinea pig red blood cells (RBC) and for hemagglutination (HA) of human type 0, guinea pig, chicken and Beagle RBC at 4°C, 22°C and 37°C. Hemadsorbing and hemagglutinating virus isolates were identified by hemadsorption inhibition (HAdI) and hemagglutination inhibition (HAI) tests with specific antisera.**

Properties of unidentified viruses

The physical and chemical properties of viruses that could not be readily identified were determined by procedures summarized by Hilleman[7].

Examination of sera

Because of extensive studies by other researchers on the blood chemistry and hematology of the dogs in this study it was not always possible to obtain sera from each dog at desired intervals. Whenever possible sera collected from dogs were tested for NT antibody against the virus isolated from that animal or a known virus of the same type.

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* Obtained from Microbiological Associates, Bethesda, Maryland

** Obtained from Microbiological Associates, Bethesda, Maryland and Flow Laboratories, Inglewood, California.

Neutralization tests were carried out with approximately 100 TCD_{50} of virus per 0.1 ml of suspension and two-fold serial dilutions of sera mixed in equal volumes and incubated for 2 hours at 22°C. All sera were inactivated at 56°C for 30 minutes.

RESULTS

A total of 164 CPE producing viruses were isolated and identified from 1602 rectal, nasal and throat swabs collected at intervals from three groups of Beagles. Four additional isolates were recovered but could not be subcultured and identified after the original isolation.

Six viruses produced CPE and HA of certain RBC but could not be readily identified by virus NT and HAI tests with specific antisera. The six viruses were apparently identical since antisera prepared against one of the viruses neutralized the remaining five. These viruses possessed properties typical for enteroviruses, i.e., a particle size of less than 50 millimicrons, inactivated by 56°C for 30 minutes, heat stable in the presence of 1 M Mg^{++}, stable at pH 3.0, not inactivated by ether, ribonucleic acid containing virus as determined by acridine orange staining and lack of viral inhibition by 5-fluorodeoxyuridine, non-pathogenic for newborn mice and hemagglutination of human type 0 and rat RBC's but not chicken or guinea pig RBC's.

Group I - 35 μCi/kg ^{90}Sr

Coxsackievirus B1 was prevalent among both the control and experimental dogs in this group (Table 1) prior to and for several weeks after inhalation of ^{90}Sr. This virus was recovered repeatedly from all 16 dogs for periods up to 10 months after the first isolations. There was no significant difference in the frequency with which the coxsackievirus B1 was isolated from the irradiated as compared to the control dogs. Coxsackievirus B5 was recovered once from a control dog and twice from swabs collected a month apart from an irradiated dog. From the 10th week through the 7th month of sampling several isolations of echovirus 6 were made with a significantly greater number

TABLE 1

Isolation of coxsackievirus B1 and B5 and echovirus 6 from Beagle dogs with a mean initial whole body burden of 35 $\mu Ci^{90}Sr$/kg and from control dogs.

Time Post Irradiation	Irradiated Dogs								Control Dogs							
	Fd13	FD17	FD23	FD82	1C	FD14	FD19	FD83	1E	FD88	FD84	FD24	1D	FD11	FD18	FD20
Pre-exposure	B1	B1	B1	B1	B1	B1	-	B1	B1	B1	B1	-	B1	-	B1	B1
1 week	B1	B1	B1	B1	-	-	B1	B1	B1	B1	B1	B1	B1	-	B1	B1
2 weeks	B1	B1	-	B1	B1	-	B1	B1	-	-	-	-	-	B1	B1	B1
3 weeks	-	B1	B1	B1	B1	B1	-	-	-	-	-	-	B1	B1	-	B1
5 weeks	-	B1	B1	B1	-	B1	B1	-	B1	B1	B1	B1	B1	-	-	-
7 weeks	-	-	-	-	B1	B1	-	B1	-	B1	-	-	-	B1	B1	-
9 weeks	-	-	-	-	B1	-	B1	B1	-	-	B1	-	B1	B1	B1	B1
10 weeks	B1	B1	B1	B1E6	-	-	-	-	-	B1	B1	B1	-	-	-	-
14 weeks	B1	B1	-	-	-	-	-	-	B1	-	B1	-	-	-	-	-
5 months	-	B1	B5	-	-	-	E6	E6	-	-	-	-	-	-	E6	-
6 months	-	-	B5	-	-	B1	-	E6	E6	E6B1	E6	E6	E6	E6B5	E6	-
7 months	-	-	-	-	-	-	-	-	-	-	-	E6	-	E6	-	-
8 months	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9 months	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 months	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11 months	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = No virus isolation

B1 = Coxsackievirus B1; B5 = Coxsackievirus B5; E6 = Echovirus 6

being recovered from the control dogs. At the time of these isolations the dogs had all been housed individually in metabolism cages in two separate rooms with an equal number of control and experimental animals in each room after having been exposed to inhalation of ^{90}Sr five to seven months previously.

Several sera from each dog collected at various intervals prior to and after the isolations of the different viruses were tested for NT antibody against coxsackieviruses B1, B2, B3, B4, B5 and B6 and echovirus 6 of human origin. All sera were negative at a dilution of 1:2.

Group II - 80 μCi/kg ^{90}Sr

Comparatively few isolations of viruses were made from this group of dogs (Table 2). Only one isolation of echovirus 6 was made from the pre-exposure samples. From the 2nd through the 4th weeks after exposure to inhalation of ^{90}Sr five isolations of an unidentified enterovirus were made from the control dogs while none were recovered from the exposed dogs. One of the coxsackieviruses, (B1, B3 or B5) was isolated from every control and experimental dog during the 7th month after exposure. All dogs in this group were housed individually in metabolism cages for the first 4 to 5 weeks after exposure and were then placed in kennel runs. No sera were available from this group of dogs to test for NT antibodies against the viruses isolated from these animals.

Group III - 140 μCi/kg ^{144}Ce

During the 5 weeks that these dogs were studied all of the types of viruses that had been isolated from the other two groups were isolated at least once from one or more of these animals (Table 3). There appeared to be a random distribution of the virus isolates among the control and exposed animals. These animals were caged individually for the duration of the study after exposure to inhalation of ^{144}Ce.

TABLE 2

Isolation of coxsackievirus types B1, B3 and B5, echovirus type 6 and an unknown enterovirus from Beagle dogs with a mean initial whole body burden of 80 μCi ^{90}Sr/kg and from control dogs.

Time Post Irradiation	Dog Number									
	Irradiated					Control				
	19B	19C	19D	22A	22E	22F	23B	19A	21C	22D
Pre-exposure	-	-	-	-	-	-	-	E6	-	-
1 week	-	-	-	-	-	-	-	-	-	-
2 weeks	-	-	-	-	-	-	-	-	*	-
3 weeks	-	-	-	-	-	*	-	*	*	-
4 weeks	-	-	-	-	-	-	-	-	*	-
5-6 weeks	-	-	-	-	-	-	-	-	-	-
2-6 months	-	-	-	-	-	-	-	-	-	-
7 months	B5	B5	B3	B1	B5	B5	B5	B5	B5	B3
8-11 months	-	-	-	-	-	-	-	-	-	-

* = Unidentified virus isolated.

\- = No viruses isolated.

B1 = Coxsackievirus type B1

B3 = Cosackievirus type B3

B5 = Coxsackievirus type B5

E6 = Echovirus type 6

TABLE 3

Isolation of coxsackieviruses types B1, B3 and B5 and echovirus type 6 and an unknown echovirus from Beagle dogs with a mean initial whole body burden of 140 $\mu Ci^{144}Ce/kg$.

	Dog Number											
	Irradiated						Control					
	60B	60C	60D	62B	62F	63C	53A	54C	56A	60A	61C	62A
Pre-Exposure	-	-	-	-	-	-	-	-	-	-	-	-
1 week	-	-	-	-	-	-	-	-	-	-	-	-
2 weeks	-	-	-	-	-	E6	-	E6	-	-	*	-
3 weeks	-	-	B1	B3	-	B5	B3	-	-	-	B5	-
4 weeks	-	-	-	-	-	-	-	-	-	B5	-	-

* = Unknown virus isolated

\- = No virus isolated

B1 = Coxsackievirus B1

B3 = Coxsackievirus B3

B5 = Coxsackievirus B5

E6 = Echovirus 6

Sera collected from these dogs before, during and after the time that they were sampled for viruses were tested for NT antibody against the viruses isolated from the individual dogs. As seen by the data summarized in Table 4, NT antibody against the virus isolates was present in only part of the dogs. In some animals the antibody was present before the virus was isolated. Neutralizing antibody against coxsackievirus B3 and B5 were observed but none against the coxsackievirus B1, echovirus 6 or one of the unknown virus isolates. Neutralizing antibody titers ranged from 1:4 (the lowest dilution tested) to 1:16 .

During the course of this study there were no overt symptoms of illness that could be attributed directly to infection with any of the viruses isolated from the dogs in any group.

Many hemagglutinating and hemadsorbing virus isolates were obtained from the MK cell cultures. All were identified by HAI and HAdI tests as Simian virus (SV)5, a common contaminant of MK cell cultures. No myxoviruses were recovered from the dog specimens.

The comparative efficiency of the various cell culture systems used to isolate the viruses recovered have been summarized in Table 5. The number of passages required to establish each isolate is also summarized. Approximately 3 times as many isolations of coxsackievirus B1 were made in Hela cell cultures as in MK cells and only one isolate was recovered in both cell culture systems. All four of the coxsackievirus B3 isolations were made in Hela cells and 12 of the 14 coxsackievirus B5 isolates were recovered in Hela cells. All 26 of the echovirus 6 and unknown viruses were recovered in MK cell culture and none in Hela cells. No isolations of virus were made in DK or DL cell cultures although preliminary observations had suggested that a virus might be present in some of these cultures.

TABLE 4

Neutralizing antibody titres of dogs from which each enterovirus was isolated. Exposed dogs had a mean initial whole body burden of 140 $\mu Ci\ ^{144}Ce/kg$.

Time Post Exposure In Weeks	Control Dogs					Exposed Dogs			
	53C	54C	60A	61C	61C	62B	63C	63C	60D
	Virus Isolates								
	B3	E6	B5	B5	*	B3	E6	B5	B1
2-pre exposure	< 1:4	< 1:4	1:8	1:8	< 1:4	< 1:4	< 1:4	< 1:4	< 1:4
1	< 1:4	< 1:4	1:8	1:4	< 1:4	< 1:4	< 1:4	1:4	< 1:4
2	1:4	< 1:4	1:8	1:8	< 1:4	< 1:4	< 1:4	< 1:4	< 1:4
3	1:4	< 1:4	1:8	1:8	< 1:4	< 1:4	< 1:4	< 1:4	< 1:4
5	1:4**	< 1:4	1:4	1:8	< 1:4**	< 1:4**	< 1:4	< 1:4**	< 1:4**
7	< 1:4	< 1:4	1:4	1:8**	< 1:4	< 1:4	< 1:4	< 1:4	< 1:4
11	1:8	< 1:4	1:8	1:8	< 1:4	< 1:4	< 1:4	1:4	< 1:4
15	1:4	< 1:4	1:4	1:8	< 1:4	< 1:4	< 1:4	< 1:4	< 1:4
20	1:4	< 1:4	1:8**	1:8	< 1:4	< 1:4	< 1:4	< 1:4	< 1:4

* = Unknown virus
** = First serum sample collected after isolation of virus
B3 = Coxsackievirus type B3
B5 = Coxsackievirus type B5
E6 = Echovirus type 6.

TABLE 5

Comparative sensitivity of HeLa and primary Rhesus monkey kidney (MK) cell cultures for Isolation of enteroviruses from Beagle dogs.

Virus Isolates	Total Isolations	Number of isolates and passages required for isolation in*							
		Hela				MK			
		1	2	3	%	1	2	3	%
Coxsackievirus B1	117	50**	28	5	71	25	9**	1	29
Coxsackievirus B3	4	1	3	0	100	0	0	0	0
Coxsackievirus B5	14	8	4	0	86	0	0	2	14
Echovirus 6	23	0	0	0	0	3	5	15	100
Unknown enterovirus	6	0	0	0	0	3	2	1	100

* No viruses were isolated in either DK or DL cell cultures.

** One isolation of coxsackievirus B1 was made in both HeLa and MK from one sample.

The sources of the swabs from all three groups of dogs from which viruses were isolated have been summarized in Table 6. There was little difference in the number of isolations of the coxsackieviruses made from a given source when the control and exposed Beagles were compared but these viruses were recovered more frequently from rectal swabs than from nasal and throat swabs. The echoviruses were recovered more frequently from the control than from the exposed dogs and occurred about as often in swabs taken from one source as from another.

Because there was no significant difference in the frequency with which the coxsackie viruses were isolated from the different groups of dogs, all isolations were considered in relation to seasonal occurrence (Fig. 1). The echovirus isolates were also considered on this basis although they occurred more frequently in control than in experimental dogs. These data were tabulated as the per cent of positive dogs sampled per month during the study period. There was a definite tendency toward a cyclic occurrence of these viruses in Beagles, with coxsackieviruses occurring most frequently in the fall of 1964 and winter of 1965 and again during the winter and spring of 1966. The echoviruses were most prevalent in the winter and summer of 1965 and late winter of 1966.

DISCUSSION

Most investigations for the presence of viruses in animals have been made by inoculating material from swabs or tissues into cultures originating from the species from which the specimens were taken. Gelfand and Flynn[8] made an extensive study of the feces of dogs using dog kidney cell cultures for isolation of viruses and recovered only infectious canine hepatitis virus. Ablett et al[9] and Spaulding et al[10] also isolated this virus from puppies using dog kidney. Dog kidney tissue cultures were also

TABLE 6

Sources of enteroviruses isolated from Beagles exposed to aerosols of ^{90}Sr and ^{144}Ce and from control Beagle Dogs.*

Viruses Isolated	Exposed					Control				
	R	N	T	N+T	Total	R	N	T	N+T	Total
Coxsackie B1	26	15	14	9	64	25	8	13	8	54
Coxsackie B3	2	0	0	0	2	1	0	1	0	2
Coxsackie B5	5	0	1	0	6	4	3	1	0	8
Echo 6	1	1	2	1	5	8	4	5	0	17
Unknown	0	0	0	0	0	2	3	1	0	6
TOTAL	34	16	17	10	77	40	18	21	8	87

R = Rectal; N = Nasal, T = Throat and N+T = Pooled nasal and throat swabs.

* = A total of 801 swabs were tested from control and also from exposed Beagle dogs.

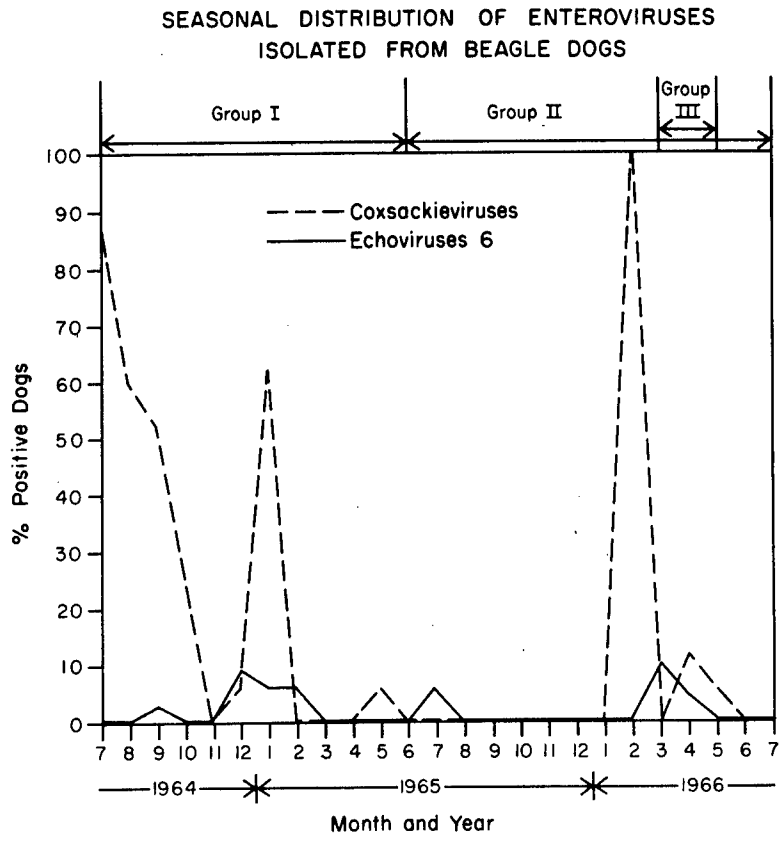
SEASONAL DISTRIBUTION OF ENTEROVIRUSES
ISOLATED FROM BEAGLE DOGS
Group I
Group II
Group III
Coxsackieviruses
Echoviruses 6
% Positive Dogs
100
90
80
70
60
50
40
30
20
10
0
7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7
1964
1965
1966
Month and Year

FIGURE 1

used by Lou and Wenner[11] to isolate reovirus 1 from dogs and by Carmichael et al[12], Stewart et al[13] and Spertzel et al[14] to isolate canine herpes-like viruses.

Pindak and Clapper[15] attempted to find viruses in canine feces by the inoculation of cell cultures other than primary dog kidney. Agents which produced CPE in monkey kidney cell cultures and in a continuous strain of canine liver cells were isolated which proved to be serologically closely related or identical to human echovirus 6. This agent produced no observable changes in dog kidney cultures. More recently Massie and Shaw[16] isolated reovirus 1 in African green monkey kidney cells from nasal, throat and rectal swabs taken from dogs.

In the present study isolations of additional agents closely related to human enteroviruses were made in rhesus monkey kidney cells from nasal, throat and rectal swabs taken from dogs.

In the present study isolations of additional agents closely related to human enteroviruses were made in rhesus monkey kidney and HeLa cells, but again none of these were isolated in dog lung or kidney cell cultures. It has previously been shown that primary dog lung and kidney cell cultures are not susceptible to infection with human type enteroviruses[17, 18]. This and the fact that they were neutralized by antiserum to the human agents suggests strongly that they are identical to the human enteroviruses.

The most frequently isolated agent was coxsackievirus B1, but coxsackieviruses B3 and B5, echovirus 6 and unidentified enterovirus were also found. These viruses were apparently carried without evidence of illness in the dogs, and were sometimes found in the same animal repeatedly during several months. There were periods of greater prevalence throughout the studies made on the 3 different groups of dogs. It is possible that this might correspond to times when human handlers were infected with one of the viruses. The recovery of human enteroviruses in the nose, throat and rectum of

Beagles further suggests the possibility that the dog may serve as an intermediate carrier for most of the enteroviruses which are common to man and often cause illness in man.

The primary purpose of this study was to determine what effect exposure to radioactive aerosols would have on the frequency of occurrence of any virus. Exposure to ^{90}Sr and ^{144}Ce had no effect on the frequency with which the coxsackie B viruses were isolated from nose, throat or rectum. However, the echoviruses were isolated much more often from controls than from the experimental animals. This was true for echovirus 6 which was isolated 10 times from 7 of 8 controls, during the 5th to 7th month after exposure, while the virus was recovered 4 times from 3 of the 8 animals exposed to the lower dose of ^{90}Sr. During the 2nd to 4th week after exposure of the dogs to the lower dose of ^{90}Sr, 5 isolations of an unidentified echovirus were made from 3 of 5 control dogs. No isolations of this virus were made in the irradiated animals. It may be postulated that the continued irradiation from ^{90}Sr might have brought about physiological changes that were not conducive to establishment of echovirus infection.

It might have been expected that antibodies to the viruses isolated could be found in the serum of the infected dogs. However, no antibodies to coxsackievirus B1, echovirus 6 or one of the unknown viruses, were found in serum collected 2 weeks after the first recovery of the viruses from the dogs. Examination of selected serum from other dogs from which coxsackie viruses B3 and B5 were isolated showed homotopic antibodies in titres of 1:4 to 1:16. Antibodies to human disease producing viruses in dogs have been reported before. These include low titres of neutralizing antibodies to polioviruses types 1 and 3, coxsackievirus A9 and B2 and echoviruses 6, 7, 8, 9, and 12 [19]. Carmichael and Barnes[20] found complement fixing antibodies to adenoviruses and Lou and Wenner[11] and Rosen[21] found antibodies to type 1 reovirus.

On the other hand, Pindka and Clapper[22] found no antibodies in dogs experimentally infected by oral ingestion of echovirus 6, although the virus was recovered from the feces of 2 of the animals 28 days after the feeding and from the blood of another 2 days after feeding. High titres of neutralizing antibodies were produced by intramuscular injection of echovirus 6.

The findings in a recent study of enteroviruses isolated from rhesus monkeys are remarkably similar to our results with dogs[23]. Simian enteroviruses were isolated from rectal swabs from nearly all of the monkeys and were isolated sporadically throughout the 11 week study period. Serum antibody was found in the monkeys but there was no correlation with the isolation of the virus. It is possible that in both monkeys and dogs, enterovirus infection is largely confined to the intestinal tract. Resistance to clinical disease may be due to cellular resistance in the intestine and not to detectalbe humoral antibodies. It is also possible that the viruses isolated may be of low virulence for the dog, since symptoms of disease were not present. The feeding of rather large doses of echovirus 6 of human origin, has been reported to produce mild and transient symptoms in only part of the challenged animals[22]. However, chronic irradiation from deposited radioactive material may change the natural defense mechanisms so that a viral infection can develop producing disease and/or death in the animal. Isolation of these viruses from the sick or dead animals exposed to radioactive material by inoculation would give more definitive answers to this question. Experiments of this kind are in progress.

Although a cyclic appearance of the viruses was observed, it was not correlated with any season of the year. This cyclic occurrence may be due to a decline in specific resistance of the dogs, variation in the strains of viruses, or a chance increase in exposure from exogenous sources.

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